

USE OF NERVE GROWTH FACTOR FOR THERAPY OF INTRAOCULAR TISSUE PATHOLOGIES

Experimental Report on the Passage of NGF through the Ocular Tissues

We used two techniques to evaluate the passage to the retina and optic nerve of different concentrations of NGF eye drops administered in the conjunctival fornix.

First we used autoradiography by means of ^{125}I -NGF incorporation. ^{125}I -NGF was radioiodinated with Na ^{125}I (Amersham) by the chloramine-T procedure and purified by Sephadex G-25 column chromatography. The specific activity was 1.0-1.5 Ci/mmol. 10 μl of ^{125}I -NGF eye drop at four different concentrations (1 $\mu\text{g}/\text{ml}$, 10 $\mu\text{g}/\text{ml}$, 200 $\mu\text{g}/\text{ml}$, and 500 $\mu\text{g}/\text{ml}$) was administered in the conjunctival fornix of twelve rabbits (3 animals for each concentration). The latter were and sacrificed 2, 6 and 24 hours after the NGF administration. The eyes were removed and fixed in 4% paraformaldehyde in phosphate buffer 100mM, pH 7.4. The eyes subsequently were placed in a 30% buffered sucrose solution for 24hrs. Sections of eyes (10 μm thickness) were cut and used for autoradiography. Briefly, slides were coated with nuclear tracking emulsion Ilford K2 (Ilford), developed using Kodak D19 developer after 1 month exposure and counterstained with Toluidine blue.

Our data show that labeled NGF was observed in the optic nerve 2 hours after the conjunctival administration when administered at a concentration of 10 $\mu\text{g}/\text{ml}$, 200 $\mu\text{g}/\text{ml}$ and 500 $\mu\text{g}/\text{ml}$. Labeled NGF increased 6 hours after the administration and was no more present 24 hours later. In the retina labeled NGF was observed only 6 hours after conjunctival administration at the same

concentration observed for optic nerve (10 $\mu\text{g}/\text{ml}$, 200 $\mu\text{g}/\text{ml}$ and 500 $\mu\text{g}/\text{ml}$).

No evidence of a passage of labeled NGF in the retina or optic nerve was observed when the eye drop contained the lower concentration of 1 $\mu\text{g}/\text{ml}$ of NGF.

This data was confirmed by ELISA technique. NGF eye drop at four different concentrations (1 $\mu\text{g}/\text{ml}$, 10 $\mu\text{g}/\text{ml}$, 200 $\mu\text{g}/\text{ml}$, and 500 $\mu\text{g}/\text{ml}$) was administered in the conjunctival fornix of sixty rabbits and the animals were sacrificed 2, 6 and 24 hours after the administration.

Our results show that NGF administration in the conjunctival fornix reached the retina and the optic nerve 2 hours after the administration and returned to the baseline values at 24 hours (see figures 1 and 2). The levels of NGF were increased in the retina and optic nerve depending on the concentration of the eye drop.

No effect was observed for the lower concentration (1 $\mu\text{g}/\text{ml}$) at any time point in both retina and optic nerve. This last observation is in line with the well known activity of NGF. In fact the active concentration of NGF for *in vitro* culture is around 250 ng/ml while the NGF is used at a concentration of approx. 0.1-1 $\mu\text{g}/\text{ml}$ in animal study using local injection (intracerebral, intraocular injection). If it is considered the eye drop administration of NGF to reach the posterior segment it is likely to hypothesize a dramatic decrease of the NGF reaching the retina and optic nerve, as shown by our study.

Therefore the concentration of 0.02 and 0.04 $\mu\text{g}/\text{ml}$, used by Okamoto in his *in vivo* study, appears to be completely unable to induce any detectable effects, in line with our data showing no activity of eye drop with a concentration at least 25 times higher (1 $\mu\text{g}/\text{ml}$).

optic nerve



